MICHAELIS-MENTEN KINETICS

In this experiment you will explore Michaelis-Menten kinetics through a hypothesis-driven approach. In Part I, you will determine the Michaelis-Menten parameters of an enzymatic cleavage reaction, and in Part II, you will design an experiment to test an inhibitor of your choice to determine if it is reversible or irreversible, and the appropriate kinetic parameters for the determined type.

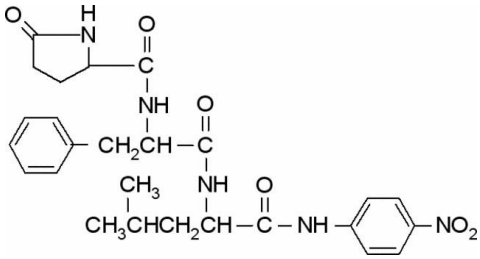
A. Background

In their seminal 1913 paper, Leonor Michaelis and Maud L. Menten described the kinetics of enzymes through the following equation,

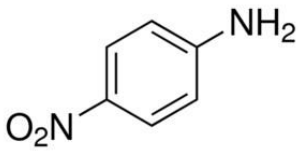
$$v = \frac{V_{max}[S]}{K_m + [S]}$$

where *v* is the rate of conversion (or reaction velocity), V_{max} is the maximum rate of conversion/reaction velocity at enzyme saturation, [S] is the substrate concentration, and K_m is the Michaelis-Menten constant.[1]

For this experiment, you will use papain as your model enzyme. Papain is a cysteine protease, meaning it is an enzyme which cleaves other proteins at a specific sequence, using cysteine at its active site. In order to measure the Michaelis-Menten parameters for this enzyme, we will use an artificial chromogenic substrate, pGlu-Phe-Leu p-nitroanilide (PFLNA):



The enzyme cleaves at the lower-right carbonyl, yielding a colored product, *p*-nitroaniline.



This product absorbs at 410 nm, and thus can be measured by UV-Vis spectroscopy.

B. Pre-Lab Investigation

This is a list of things you should research and be familiar with ***BEFORE*** you arrive in lab. Failure to adequately prepare for this lab may jeopardize your ability to complete the experiment in the allotted time.

1. Enzyme Inhibition Types (Reversible, Irreversible, Competitive, Noncompetitive, Uncompetitive)
2. Enzyme Inhibition Parameters (IC_{50}, K_i)
3. Regression Methods for Michaelis-Menten Kinetics (See Part I procedures for suggestions)
4. Preliminary plan for how to complete Part II

C. Procedure

Part I

Base Procedure:

Measure out 3 mg of crude papain into a microcentrifuge tube and add 1 mL of 50 mM pH 7.4 phosphate buffer. Vortex the sample for 3 minutes, and centrifuge for 1 minute. Separate the sample into 250 uL aliquots and dilute each to 997 µL. Add 3 µL of PFLNA (0.05 M) to the dilute aliquot, and quickly transfer 1 mL of the dilute aliquot with substrate to a cuvette and start monitoring at 410 nm for 2 minutes using the Labquest device.

Use the initial rate of reaction and the substrate concentration in your measured sample to determine V_{max} and K_m. You will need to do multiple samples changing the appropriate variables from the base procedure to complete the experiment. You may use any regression you choose, but you will be expected to justify your choice. Some suggestions for regression methods (some are better than others) are:

- Lineweaver-Burk
- Hanes-Woolf
- Eadie-Hofstead
- Direct Line – Eisenthal and Cornish-Bowden
- Nonlinear Regression

Part II

In this part of the lab, you will select one of the inhibitors provided. You will be expected to design an experiment to test **ONE** of these inhibitors and answer the following questions.

1. Is this substance an inhibitor of papain?
2. What type of inhibitor is it? (Reversible, Irreversible, Competitive, Noncompetitive, Uncompetitive)
3. What are the kinetic parameters of this inhibition? (K_i, IC_{50}, for irreversible inhibitors - equivalents needed to fully inhibit)

Do not be misled; although the procedures *section* for Part II is notably shorter, the procedures take much longer. Come prepared with an idea of how to test the inhibitor to answer these questions!

D. References

(1) Michaelis, L.; Menten, M. L. Die kinetik der invertinwirkung. *Biochem. z* **1913**, *49* (333-369), 352.